(1980)). That system also involves binding a ligand to a solid support, then exposing that support to an antibody to the ligand and then to labeled protein A. Ligand in solution can compete with bound ligand for the antibody binding sites, and therefore, the amount of labeled protein A that binds to the solid support is an indirect measure of the concentration of ligand in solution.

In each of the above-described procedures, it is necessary for the antigen to be bound to the solid support initially, so that only one IgG antibody need be employed. Erlich, Henry A., et al., *Methods in Enzymology,* 68, 443–453 (1979) describe a direct immunoassay for products translated from cloned DNA fragments, which employs radio-labeled protein A as a tracer. In this method, an antibody is immobilized on a solid support and a sample containing an antigen to the antibody is exposed to the support under conditions that permit the antigen to bind to the antibody and thereby be immobilized. The solid support is then exposed to a second antibody which binds to the immobilized antigen, and finally the solid support is exposed to the radiolabeled protein A which binds to the F_c portion of the second antibody. In the described procedure, the first antibody is digested with the proteolytic enzyme, pepsin, to remove its F_c portion prior to attachment to the solid support. The reason for this digestion is to eliminate the possibility that the labeled protein A will bind to the first antibody, and thereby create a high level of background signal.

A need exists for a method of conducting direct, two site immunoassays, using labeled binding protein as a tracer, without requiring the removal of the F_c portion of either of the antibodies.

SUMMARY OF THE INVENTION

In accordance with the present invention, a method for quantitatively determining the presence of a polyvalent ligand in an aqueous fluid involves the steps of

(a) incubating said fluid with a solid support having first antibody to said ligand bound thereto to form an immobilized antibody-ligand complex;

(b) incubating said immobilized-antibody-ligand complex with a solution containing a soluble complex of a second antibody to said ligand and a labeled binding protein that specifically binds to the F_c portion of said second antibody;

(c) separating unbound antibody and labeled binding protein from the solid support; and

(d) determining the presence of labeled binding protein either bound to said solid support or remaining in solution as a measure of the concentration of the ligand in the aqueous fluid;

whereby said method is further characterized in that the solution of second antibody-labeled binding protein complex contains an excess concentration of second antibody to provide a substantially linear relationship between the amount of bound labeled binding protein and the concentration of the ligand in the aqueous fluid, while maintaining a low level of background signal.

In a particular embodiment, prior to immobilization of the first antibody on the solid support, it can be enzymatically digested to remove the Fc binding fragment. In this embodiment, the second antibody-labeled binding protein complex is preferably constructed at or near stoichiometric equivalence (i.e., molar ratio of second antibody to labeled binding protein is about 2:1).

DETAILED DESCRIPTION OF THE INVENTION

The present invention involves a direct, two-site immunoassay employing labeled binding protein as a tracer, in which a first antibody can be immobilized on a solid support in either intact or digested form. It has been discovered that the need for enzymatically removing the F_c portion of the first antibody in such an assay is obviated, if a complex composed of labeled binding protein and soluble second antibody is formed in solution, as hereinafter described.

The general assay system involves immobilizing a first antibody to the ligand of interest on a solid support. The solid support is then exposed to a standard or sample solution which contains or is suspected of containing the ligand. An excess of binding sites are present on the solid support, so that substantially all of the ligand in the standard or sample solution binds with the immobilized first antibody. The remaining solution is then advantageously washed or otherwise removed from the solid support prior to succeeding steps. The solid support is then exposed to a solution containing a soluble complex of the second antibody to the ligand of interest and a labeled binding protein, preferably protein A, which is bound to the Fc portion of the second antibody. This complex binds to the immobilized ligand in an amount directly proportional to the amount of ligand present on the support surface. Unbound labeled binding protein-antibody complex may then be washed from the solid support, and either the amount of labeled binding protein immobilized on the support or the amount of labeled binding protein remaining in solution can be determined, and that amount can be correlated to the concentration of ligand in the original solution.

Since the first antibody can be an intact IgG immunoglobulin to which the labeled binding protein has a binding affinity, this scheme is somewhat complicated by side reactions. It has been found that free binding protein will combine with the first antibody immobilized on the solid support, whether or not the immobilized antibody has reacted with the ligand. These false binding events result in an unacceptably high background signal. For this reason, previous attempts to develop direct two-site immunoassays employing protein A, have required the enzymatic digestion of the first antibody to remove its Fc portion prior to immobilization.

It has now been found that when the labeled binding protein for such an immunoassay is provided as a soluble complex with the second antibody, these false binding events can be significantly reduced. To maintain the labeled binding protein in the complexed, rather than free form, an excess of second antibody is provided in the solution of labeled binding protein-second antibody complex. When very high concentrations of soluble second antibody (relative to the concentration of labeled binding protein) are employed, false binding events can be substantially eliminated, thereby reducing background to a very low level. However, in such a situation, the sensitivity of the reaction also suffers. At the opposite extreme, when very low concentrations of soluble second antibody are employed there is a substantial tendency for labeled binding protein to bind not only to the second antibody, but also to the immobilized first antibody. This situation results in a non-linear response-concentration relationship, and also introduces an unacceptable level of background signal due to false